PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

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PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing *(day/month/year)*	03-07-2008
Applicant's or agent's file reference: 56885-CIP3-WO	FOR FURTHER ACTION See paragraph 2 below
International application No.: PCT/IB2006/004236	International filing date *(day/month/year)*: 25-10-2006
Priority date *(day/month/year)*	
International Patent Classification (IPC) or both national classification and IPC: See Supplemental Box	
Applicant: Cellectrion AB et al	

1. This opinion contains indications relating to the following items:

- [x] Box No. I Basis of the opinion
- [x] Box No. II Priority
- [ ] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- [ ] Box No. IV Lack of unity of invention
- [x] Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- [ ] Box No. VI Certain documents cited
- [ ] Box No. VII Certain defects in the international application
- [ ] Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further opinions, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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International application No.
PCT/IB2006/004236

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Cover sheet

International patent classification (IPC)
G01N33/487(2006.01)
B81B7/02(2006.01)
G01N27/00(2006.01)

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International application No.
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Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material

      ☐ a sequence listing

      ☐ table(s) related to the sequence listing

   b. format of material

      ☐ on paper

      ☐ in electronic form

   c. time of filing/furnishing

      ☐ contained in the international application as filed.

      ☐ filed together with the international application in electronic form.

      ☐ furnished subsequently to this Authority for the purposes of search.

4. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

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Box No. II Priority

1. ☐ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.

2. ☒ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

The priority claim is considered not to have been made because the international application has an international filing date which is later than the date on which the priority period expired. It is also later than the period of two months from the date according to the request for restoration of right to priority. See PCT Rule 26bis.2. See also PCT Form PCT/RO/111 which was sent to the applicant from the International Bureau on 21 May 2008.

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Box No. V **Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	61, 67-69	YES
	Claims	1-60, 62-66, 70-73	NO
Inventive step (IS)	Claims		YES
	Claims	1-73	NO
Industrial applicability (IA)	Claims	1-73	YES
	Claims		NO

2. Citations and explanations:

The present application relates to microfluidic systems and methods for altering the solution environment around a nanoscopic or microscopic object, such as a sensor, and methods for modulating or studying receptors. According to the Applicant, traditional patch-clamp methods for measuring ion channel activity in cells have not been the methods of choice for developing high-throughput screening (HTS) platforms, since these methods lack the ability to introduce test compounds onto cells in a controlled, rapid and parallel fashion. The solution to this problem as presented in this application includes applying minimised time intervals between sample deliveries, e.g. on the order of microseconds and seconds, which permits rapid analysis of compounds, e.g. drugs.

The following relevant document is cited in the international search report:

D1: WO2006074350 A2

In document D1, the same problem regarding patch-clamp methods is described as in the present application and the same solution of rapidly switching the solution environment is disclosed. See page 2, lines 10-16.

D1 discloses microfluidic systems and methods that can be applied in any sensor technology in which the sensing element needs to be exposed rapidly, sequentially, and controllably, to a large number of different solution environments whose characteristics may be known or unknown.

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In contrast to prior art microfluidic systems, the interval between sample deliveries is minimized, e.g., on the order of microseconds and seconds, permitting rapid analysis of compounds (e.g., drugs). The system and methods may be used for modulating, controlling, preparing or studying receptors.

The system in D1 comprises a substrate for changing a solution environment around a sensor, the substrate comprising a plurality of channels, each channel comprising an outlet; and a scanning mechanism for selectively exposing a sensor to a fluid stream from an outlet, wherein each of the channels delivers a fluid stream into the open volume chamber. Alternatively, the system comprises an open-volume chamber for receiving a sensor; and a plurality of channels, each channel comprising an outlet for delivering a substantially separate fluid stream into the chamber, wherein each of the channels delivers a fluid stream into the open volume chamber. See page 2, line 19 - page 3, line 5, claims 1, 13 and 19.

The plurality of sample delivery channels intersect with a first channel which is also connected to a buffer reservoir and to a chamber for receiving a sensor. Rapid flow of solution through the first channel and/or sample channels can be achieved through a positive pressure mechanism in communication with the buffer reservoir and/or sample channels. See page 10, lines 18-26.

The plurality of sample channels may intersect with a central "spine" channel which feeds sample into the sensor chamber, see figures 14 and 15 and page 23, lines 7-18. This embodiment including a central channel is considered to correspond to the delivery channel according to claim 1 of the present application.

The system comprises a mechanism for holding a sensor, which is coupled or connected to a positioner for positioning the sensor in proximity to an outlet of a channel (page 3, lines 22-24, page 4, lines 1-2). The mechanism may be a patch clamp pipette, a capillary or a hollow electrode (page 28, lines 23-27).

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The sensor may be a cell, such as a patch-clamped cell, and the cell may comprise an ion channel, such as a G-protein coupled receptor, which is to be studied (claims 27, 32 and 33). Fluorescence or electrochemistry may be used in the method of detection (page 32, lines 27-33).

The sample applied to the sensor may comprise drug candidates, such as agonists or antagonists (claims 29-31).

Thus, the subject-matter of claims 1-4, 31-35, 44-46 and 60 lacks novelty and inventive step.

The system comprises a mechanism for varying pressure across channels. See page 3, lines 16-19, page 4, lines 8-10 and claim 23. The sensor is scanned sequentially across the at least two aqueous fluid streams, thereby altering the aqueous solution environment around the object. Scanning can be mediated by pressure drops applied to the channels (page 15, line 32 - page 16, line 2).

Thus, the subject-matter of claims 5-9, 30, 36-39, 47 and 64 lacks novelty and inventive step.

Further, the subject-matter of claim 62 may lack novelty in view of the above described technical features. The claim definitely lacks inventive step.

The patch-clamped cell may be positioned relative to the delivery channel outlets using a patch clamp pipette coupled or connected to a positioner (claim 28).

The subject-matter of claims 10, 11, 41, 42 and 63 is therefore considered to lack novelty. Inventive step is definitely lacking for the embodiments according to claims 10, 11, 41, 42 and 63, since they are obvious to a person skilled in the art.

Among voltage clamp techniques, patch clamp is most suitable for measuring currents in the pA range. The low noise property of patch clamp is achieved by tightly sealing a glass microelectrode or patch clamp pipette onto the plasma membrane of an intact cell thereby producing an isolated patch.

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The resistance between the pipette and the plasma membrane is critical to minimize background noise and should be in excess of 1x10(9) ohm (1 giga-ohm) to form a "giga seal" (page 62, line 28 - page 63, line 2 and claim 37).

Therefore, the subject-matter of claims 12, 13, 40 and 43 lacks novelty.

Further, the subject-matter of claims 14-26 lacks novelty in view of what is disclosed in claims 2-19 of D1.

The method in D1 may be used for studying the memory properties of a receptor. The memory functions may be short-term, medium-term, or long-term memory functions. The effects of a drug on memory properties of a biosensor may be studied. See claims 20-22.

The subject-matter of claims 27-29 thus lacks novelty and inventive step.

The system described in D1 is a microfluidic device, and comprise microchannels and other components which are microscale-sized. See e.g. page 79, lines 3-4.

Therefore, the subject-matter of claims 48-59 probably lacks novelty. Inventive step is definitely lacking for said claims.

The method in D1 comprises rapidly changing the solution environment around a sensor. Fluid exchange may occur within less than a minute, such as so rapidly as within milliseconds or nanoseconds. See page 16, line 28 - page 17, line 2.

Therefore, the subject-matter of claims 65, 66, 70-73 lacks novelty and inventive step.

The subject-matter of claims 61, 67-69 is not specifically disclosed by document D1. However, the embodiments of said claims are considered to be obvious to a person skilled in the art and therefore lack inventive step.

The subject-matter of claims 1-73 fulfils the requirement of industrial applicability.